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Leaf structure and specific leaf mass: the alpine desert plants of the Eastern Pamirs, Tadjikistan

VLADIMIR I. PYANKOV[1], ALEXANDRA V. KONDRATCHUK[1] AND BILL SHIPLEY[2]*

[1] *Department of Plant Physiology, Urals State University, Ekaterinburg 620083, Russia*
[2] *Department of Biology, University of Sherbrooke, Sherbrooke, Quebec J1K 2R1, Canada*

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SUMMARY

This study examines interrelationships between eight leaf attributes (specific leaf mass, area, dry mass, lamina thickness, mesophyll cell number per cm^2, mesophyll cell volume, chloroplast volume, and number of chloroplasts per mesophyll cell) in field-grown plants of 94 species from the Eastern Pamir Mountains, at elevations between 3800 and 4750 m. Unlike most other mountain areas, the Eastern Pamirs, Karakorum system, Tadjikistan provide localities where low temperatures and radiation combine with moisture stress at high altitudes. For all the attributes measured, significant differences were found between plants with different mesophyll types. Leaves with dorsiventral palisade structure (dorsal palisade, ventral spongy mesophyll cells) had thicker leaves with larger but fewer mesophyll cells, containing more and larger chloroplasts. These differences in mesophyll type are reflected in differences in the total surface of mesophyll cells per unit leaf area (A_{mes}/A) or volume (A_{mes}/V). Plants with isopalisade leaf structure (palisade cells under both dorsal and ventral surfaces) are more commonly xerophytes and their increased values of A_{mes}/A and A_{mes}/V decrease CO_2 mesophyll resistance, which is an important adaptation to drought. Path analysis shows the critical importance of mesophyll cell volume in leading to the covariance between the different leaf attributes and hence to specific leaf mass (SLM), even though mesophyll cell volume is not itself strongly correlated with SLM. This is because mesophyll cell volume increases SLM through its effects on leaf thickness and chloroplast number per cell, but decreases SLM through its negative effect on mesophyll cell density.

Key words: alpine vegetation, mesophyll structure, path analysis, structural equation modelling, specific leaf area, SLA, specific leaf mass, SLM.

INTRODUCTION

Much effort has been expended in characterizing the physiological functions, for example gas exchange or the economy of nitrogen (N) and carbon (C), of leaves from species with different habitat preferences. These physiological processes occur in structures (organelles, cells, tissues) which have specific spatial relationships to one another within the leaf. Because of this, leaf anatomy can be expected to affect the physiological processes of the leaf and, ultimately, the ecological and evolutionary success of plants growing in different environments. For instance, the ratio of the surface area of the mesophyll cells to the surface area of the leaf (A_{mes} : A) is correlated with the rate of CO_2 assimilation (Nobel *et al.*, 1975; Longstreth & Nobel, 1979; Nobel & Walker, 1985; Patton & Jones, 1989). The ratio of the surface area of the chloroplasts to the surface area of the leaf (A_{chl} : A) is related to the internal resistance for CO_2 diffusion (Laisk *et al.*, 1970; Araus *et al.*, 1986; Evans *et al.*, 1994; Evans & von Caemmerer, 1996). This suggests that the size, number and layering of mesophyll cells within the leaf, as well as the size and number of chloroplasts within each mesophyll cell, could exert strong control over basic physiological functions such as photosynthesis and transpiration. Presumably, different combinations of size and number of these cells and organelles will lead to different physiological outcomes in different environments.

*Author for correspondence (fax 001 819 821 8049; e-mail bshipley@courrier.usherb.ca).

One morphological attribute of leaves that presumably includes such size–number trade-offs is specific leaf mass (SLM). The SLM is known to be correlated with relative growth rate under some environmental conditions (Poorter & Remkes, 1990); with leaf gas exchange (Mooney *et al*., 1978; Field & Mooney, 1983; Ellsworth & Reich, 1992); with seedling regeneration (Shipley *et al*., 1989; Maranon & Grubb, 1993); and with leaf palatability (Lucas & Pereira, 1990; Choong *et al*., 1992). Thus, SLM is implicated in ecological processes (growth and survival) that are key to evolutionary fitness.

The SLM may be expressed as the product of leaf thickness and leaf tissue density (Wiltkowski & Lamont, 1991; Garnier & Laurent, 1994). Leaf thickness and leaf tissue density are often themselves correlated (Shipley, 1995) and can both be affected by the spatial arrangement of cells within the leaf. For example, at a given laminar thickness, and for cells packed tightly, many small mesophyll cells in several layers would lead to a greater leaf density than fewer large mesophyll cells arranged in fewer layers. These speculations lead to the two main questions explored in this paper. How do different combinations of cell and organelle size compared with number vary between the leaves of different plant species? Is it possible to model quantitatively how size and number interact to determine SLM?

In order to answer these questions, we studied 94 species of alpine plants found on a high mountain plateau in the Eastern Pamirs. This area is distinguished from most other mountain systems by an extremely dry continental climate that has led to the formation of a habitat type called mountain deserts (Ikonnikov, 1963). The coefficient of aridity of the Eastern Pamirs is close to the aridity of hot deserts of Central Asia (Pyankov & Mokronosov, 1991). The area is characterized by low temperatures, high insolation and UV radiation, reduced partial pressure of CO_2, and a short growing season.

Previous studies of the leaf morphology and mesophyll structure of alpine species, conducted in New Zealand (Körner *et al*., 1986), the Alps (Körner & Diemer, 1994; Körner *et al*., 1989), the Caucasus (Goryshyna & Hetsuriany, 1980), and Tien-Shan (Miroslavov & Kravkina, 1990a,b, 1991), have documented the most common features of adaptation of plants to high elevations. These are small thick leaves, increased SLM, an increased number of layers of palisade cells, and larger sizes of mesophyll cells. All of these studies were carried out at elevations below 3500 m and in comparatively humid ecosystems, with annual precipitation of >700 mm. It is likely that the unique environmental conditions and high altitudes of the Eastern Pamirs have affected the structure of the photosynthetic apparatus of plants occurring in this region. Preliminary studies of plants from the Eastern Pamirs (Pyankov & Kondratchuk, 1995) have documented different types of mesophyll (dorsiventral, isopalisade, homogeneous and succulent), and quantitative parameters of leaf structure (Pyankov & Kondratchuk, 1998).

MATERIALS AND METHODS

The study was conducted in the summers of 1989 and 1990, in the vicinity of the Pamirs Biology Station of the Academy of Sciences of Tajikistan. The station is located at an altitude of 3860 m in the village of Chechekty, 25 km from the town of Murgab. The Eastern Pamirs (39° 05′–37° 20′ N, 75° 10′–75° 40′ E) belongs to the Karakorum Mountain Range formed in the quaternary period. According to floristic assignment, the Eastern Pamirs belong to the high-mountain Tibetan province of the Central Asian sub-region of the Afro-Asian desert region (Lavrenko, 1962). Summers in the Eastern Pamirs are short, dry and cold. The frost-free period at a height of 3860 m continues for 25–50 d; freezing (from -1 to -5°C) and snow may occur during this period (Ladygina & Litvinova, 1966). The average yearly temperature is *c*. -2°C, the average summer temperature is 7.6°C, and average July temperatures range between 8.2 and 11.2°C. The average low temperature in July at a height of 3860 m does not exceed 5°C, whereas the height of 4100–4200 m marks the border of nightly frosts. The annual precipitation varies between 70 and 120 mm, and in the summer months (June–Aug.) average monthly precipitation ranges from 12–20 mm. The annual number of days with air humidity $<30\%$ varies between 200 and 250. The height of snow in winter does not exceed 10 cm, and the margin of snow during summer is at 4800–5100 m. The average July temperature at 5000 m is *c*. 0°C.

Leaves were collected at elevations between 3800 and 4750 m in late June and early July, mainly at the stage of flowering. We investigated 94 species from 32 families (Table 1) occurring in three elevation belts: sub-alpine, alpine and nival (4700–5000 m). To smooth the variability in the material, five to 10 leaves were taken from five to 10 plants belonging to ecotypes typical of the species studied. The anatomical characteristics were determined according to Mokronosov (1978) and Pyankov *et al*. (1998).

The leaf thickness of laminar leaves, or the diameter of cylindrical leaves (e.g. *Gypsophila capituliflora*) or assimilatory shoots (e.g. *Ephedra regeliana*), and the size of mesophyll cells, were measured directly in the field using leaf cross-sections stored in Tris–HCl–sorbitol buffer pH 7.4, a Biolam D-13 light microscope (LOMO, Russia), and an AM-9–2 eyepiece micrometer (GSZ, Russia). Chloroplast dimensions were measured using photographs of the same cross-sections using an MFN-11 camera attachment (LOMO) and Mikrat-300 films (AO Tasma-Kholding, Russia). The photographs were

subsequently projected onto a screen and the plastid dimensions measured. To determine the number of chloroplasts in the cell and the number of cells per unit leaf area, samples were fixed with 3.5% glutaraldehyde in phosphate buffer pH 7.0. The number of cells per unit leaf area was determined from samples macerated in 20% KOH. The number of chloroplasts per cell was determined in a mixture of 5% CrO_3 with 1 M HCl after heating on a water bath at 50–60°C for 15–20 min. The cell volume in the palisade mesophyll was calculated as the volume of a cylinder with a coefficient depending on the cell length : width ratio (Tselniker, 1978). The cell volume was determined according to the Chezare relation for a rotation ellipsoid. The following equations were used:

$$S_{cell} = [\pi D(2L + D)/2]$$

$$V_{cell} = \pi(d)^2 LK$$

(S_{cell} and V_{cell} are the surface area and volume of the cell; L and D are the length and width of the cell, respectively; d is $(D/2)$; and K is the cell shape coefficient. This coefficient was found empirically (Tselniker, 1978) and has a good correlation ($r = 0.95$) with the cell length : width ratio. Specifically, $K = 0.38 + 0.117L/D$.)

Chloroplast surface and volume were calculated assuming a rotation ellipsoid geometrical model.

$$S_{chl} = 4\pi(l\ d^2)^{2/3}$$

$$V_{chl} = 4/3\ \pi\ l\ d^2$$

(S_{chl} and V_{chl} are the surface area and volume of the chloroplast; and l and d are half the length and width of the chloroplast, respectively).

Determinations of leaf area, leaf thickness and mass per unit area were obtained from 10 replicates of five to 10 samples of each species. The number of chloroplasts in cells, as well as cell and chloroplast dimensions, were determined by macerating the tissues obtained from five to 10 fixed leaf fragments of 30 replicates per species. The number of cells in macerated tissues was used to calculate the number of cells per unit leaf area, and was determined in 20 replicates of cell suspension with the use of 90 or 225 square cells of the Goryaev haemocytometer, depending on cell concentration in suspension. Chloroplast number per unit of leaf area was calculated by multiplying chloroplast number per cell by the number of cells (palisade and spongy) per unit leaf area. Total indices of the surfaces of palisade cells and chloroplasts per unit leaf area, A_{mes}/A and A_{chl}/A were calculated by multiplying the surfaces of elements (cell, chloroplast) by their number in unit leaf area. This procedure kept the standard errors of the mean below 5%.

Species were classified according to field abundance based on plant occurrence in the Eastern Pamirs (Ikonnikov, 1963). There were 27 rare, 27 occasional and 40 common species. The mesophyll type was classified for each species as follows. Dorsiventral mesophyll (dv, 46 species) is formed from two cell types, palisade and spongy. The palisade cell arrangement under the upper epidermis was in two or three layers, having a cylindrical shape with hemispheres on each end. Spongy cells (two to four layers) were on the ventral surface and had an irregular shape. Isopalisade mesophyll (ip, 24 species) had two to four layers of palisade cells under both dorsal and ventral surfaces, but some species (e.g. *Krascheninnikovia ceratoides*) had one or two layers of spongy mesophyll between them. Homogeneous mesophyll of grasses (hg, 12 species) consisted only of spongy mesophyll. The remaining 12 species had other types of mesophyll structure such as the homogenous dicot type (spongy cells only) as well as succulents and *Alliums* with a large cell volume; the latter included too few species per type to be statistically analysed. These classifications are indicated in Table 1.

Statistical analysis

In order to better approximate multivariate normality, data were transformed to their natural logarithms. The multivariate patterns of correlation in the leaf attributes were summarized using principal components analysis based on the correlation matrix in order to remove the gross size-related effects. The importance of each leaf attribute in defining each of the principal components was determined from its loading on that axis. The results were summarized using a biplot (Gabriel, 1971). This type of diagram plots the position of each species on the first two axes, and then plots the loading of each attribute on the same graph as an arrow. The length of the arrow, when projected vertically (axis 1) or horizontally (axis 2), is proportional to its correlation with that axis. Thus an arrow parallel to axis 1 would load onto axis 1 but not onto axis 2; an arrow parallel to axis 2 would load onto axis 2 but not onto axis 1; an arrow with a 45° angle to axis 1 would load equally strongly on both axes.

A more detailed model of how the variables interact as a system, and an inferential test of this multivariate hypothesis, can be obtained using structural equation modelling in the form of path models. Path models were fitted using the *EQS* package (Bentler, 1995) based on maximum likelihood techniques; goodness-of-fit was evaluated using the maximum likelihood chi-square statistic (Bollen, 1989) or the Satorra–Bentler correction of this statistic, which is more robust to non-normality of the data (Bentler, 1995). This test consists of comparing the observed covariance matrix with the covariance matrix predicted by the model. Data that contradict the predicted patterns of covariance, and

Table 1. *Ninety-four species occurring in an extremely cold mountain desert in the Eastern Pamir Mountains, taxonomy follows Czerepanov (1995)*

Species	Family	Mesophyll type	Ecological group	Rarity
Allium platyspathum	Alliaceae	al	m	r
Allium sp.	Alliaceae	al	m	r
Lomatocarpa albomarginata	Apiaceae	dv	m	r
Ajania tibetica	Asteraceae	ip	x	c
Artemisia leucotricha	Asteraceae	ip	x	c
Artemisia pamirica	Asteraceae	ip	xm	c
Artemisia rhodantha	Asteraceae	ip	x	c
Erigeron heterochaeta	Asteraceae	dv	m	c
Erigeron poncinsii	Asteraceae	ip	x	c
Leontopodium ochroleucum	Asteraceae	dv	m	c
Ligularia alpigena	Asteraceae	dv	m	r
Pyrethrum djilgense	Asteraceae	ip	xm	r
Saussurea salsa	Asteraceae	dv	xm	r
Senecio krascheninnikovii	Asteraceae	ip	x	r
Serratula procumbens	Asteraceae	ip	x	r
Taraxacum dissectum	Asteraceae	dv	m	r
Taraxacum leucantum	Asteraceae	ip	m	c
Christolea crassifolia	Brassicaceae	s	xm	c
Draba korshinskyi	Brassicaceae	h	m	c
Draba pamirica	Brassicaceae	dv	m	r
Smelowskia pectinata	Brassicaceae	ip	xm	c
Sophiopsis annua	Brassicaceae	ip	xm	r
Macrotomia euchroma	Boraginaceae	ip	xm	c
Lindelofia pterocarpa	Boraginaceae	ip	xm	c
Lonicera semenovii	Caprifoliaceae	dv	m	r
Gypsophila capituliflora	Caryophyllaceae	dv	xm	o
Silene graminifolia	Caryophyllaceae	dv	m	o
Stellaria winkleri	Caryophyllaceae	dv	m	r
Krascheninnikovia ceratoides	Chenopodiaceae	ip	x	c
Chenopodium foliosum	Chenopodiaceae	dv	xm	o
Suaeda olufsenii	Chenopodiaceae	s	xm	o
Rhodiola gelida	Crassulaceae	s	xm	o
Rhodiola heterodonta	Crassulaceae	s	xm	c
Rhodiola pamiroalaica	Crassulaceae	s	xm	c
Carex melanantha	Cyperaceae	hg	m	c
Carex orbicularis	Cyperaceae	hg	m	c
Carex pseudofoetida	Cyperaceae	hg	m	c
Carex stenocarpa	Cyperaceae	hg	m	r
Carex dimorphotheca	Cyperaceae	hg	xm	c
Kobresia capilliformis	Cyperaceae	hg	m	c
Ephedra regeliana	Ephedraceae	ip	x	o
Astragalus tibetanus	Fabaceae	ip	xm	c
Hedysarum minjanense	Fabaceae	ip	xm	c
Oxytropis chiliophylla	Fabaceae	ip	xm	c
Oxytropis incanescens	Fabaceae	ip	xm	r
Oxytropis globiflora	Fabaceae	ip	m	o
Corydalis stricta	Fumariaceae	dv	xm	o
Gentiana karelinii	Gentianaceae	h	m	c
Gentiana leucomelaena	Gentianaceae	h	m	o
Swertia marginata	Gentianaceae	h	m	c
Geranium himalayense	Geraniaceae	dv	xm	r
Dracocephalun heterophyllum	Lamiaceae	dv	xm	c
Dracocephalum paulsenii	Lamiaceae	dv	xm	c
Lloydia serotina	Liliaceae	ip	m	c
Acantholimon diapensioides	Limoniaceae	ip	x	c
Parnassia laxmannii	Parnassiaceae	dv	m	o
Plantago arachnoidea	Plantaginaceae	ip	x	o
Achnatherum splendens	Poaceae	hg	m	r
Calamagrostis anthoxanthoides	Poaceae	hg	m	c
Elymus nutans	Poaceae	hg	m	o
Hordeum turkestanicum	Poaceae	hg	xm	c
Leymus pubescens	Poaceae	hg	xm	c
Stipa orientalis	Poaceae	hg	xm	c
Oxyria digyna	Polygonaceae	dv	xm	r

Table 1 (*cont.*)

Species	Family	Mesophyll type	Ecological group	Rarity
Knorringia pamiricum	Polygonaceae	ip	xm	o
Bistorta viviparum	Polygonaceae	dv	m	c
Rheum spiciforme	Polygonaceae	dv	xm	o
Androsace akbaitalensis	Primulaceae	dv	xm	c
Glaux maritima	Primulaceae	dv	m	o
Papaver involucratum	Papaveraceae	dv	m	r
Primula algida	Primulaceae	dv	m	o
Primula macrophylla	Primulaceae	dv	m	c
Primula pamirica	Primulaceae	dv	m	r
Clematis tangutica	Ranunculaceae	dv	m	o
Halerpestes sarmentosa	Ranunculaceae	dv	m	o
Oxygraphis glacialis	Ranunculaceae	dv	m	o
Ranunculus krasnovii	Ranunculaceae	dv	m	o
Ranunculus pseudohirculus	Ranunculaceae	dv	m	c
Ranunculus rufosepalus	Ranunculaceae	dv	m	o
Comarum salesovianum	Rosaceae	dv	m	o
Pentaphylloides dryadanthoides	Rosaceae	dv	xm	o
Potentilla anserina	Rosaceae	dv	m	o
Potentilla malacotricha	Rosaceae	dv	xm	r
Potentilla moorcroftii	Rosaceae	dv	xm	o
Potentilla multifida	Rosaceae	dv	m	c
Potentilla pamirica	Rosaceae	dv	xm	o
Sibbaldia tetrandra	Rosaceae	dv	m	r
Saxifraga hirculus	Saxifragaceae	dv	m	r
Pedicularis ludwigii	Scrophulariaceae	dv	m	c
Scrophularia pamirica	Scrophulariaceae	dv	xm	o
Myricaria squamosa	Tamaricaceae	dv	xm	r
Valeriana fedtschenkoi	Valerianaceae	dv	xm	r
Viola tianschanica	Violaceae	dv	m	r
Zygophyllum rosowii	Zygophyllaceae	s	xm	r

Field occurence: r, rare; o, occasional; c, common. Ecological groups: x, xerophyte; m, mesophyte; xm, intermediate. Mesophyll type: al, *Allium*; dv, dorsiventral; ip, isopalisade; s, succulent; h, homogeneous; hg, homogeneous type of grass.

therefore the hypothesized causal structure of the data, will produce a significant chi-square value, indicating that the model must be rejected. A well-fitting model will produce a non-significant chi-square value. A more detailed explanation of this method in a biological context is given by Shipley & Meziane (1998).

RESULTS

Table 2 lists the estimated values of each leaf attribute for each of the 94 species, and Fig. 1 shows the bivariate relationships between these leaf attributes. A principal components analysis of seven leaf attributes captured 75% of the variance in only two principal components (Fig. 2). The first component (axis 1) contrasted the number of mesophyll cells per square centimetre with a set of highly correlated attributes relating to cell size: mesophyll and chloroplast volume, the number of chloroplasts per mesophyll cell, and leaf thickness. The second component (axis 2) reflects differences in leaf surface area and dry mass, thus capturing differences in SLM that are linearly uncorrelated with the other variables. Because of the loadings, species with higher SLM occur at the bottom of the graph and species with low SLM occur at the top.

There were no significant differences between the mesophytes, xerophytes and intermediates (meso/xerophytes), nor between groups defined on field abundance, in any single leaf attribute, based on non-parametric ANOVA (Kruskal–Wallis test, $P > 0.05$.) Significant differences were found when comparing species with different types of mesophyll structure (Kruskal–Wallis test, $P < 0.05$) for every variable shown in Fig. 3. Species with the dorsiventral arrangement tended to have thicker leaves with fewer but larger mesophyll cells. Because chloroplast volume did not differ between the groups, the larger mesophyll cells of species with the dorsiventral arrangement resulted in a larger number of chloroplasts per mesophyll cell (Fig. 3). The multivariate pattern (Fig. 2) largely confirms these observations, but shows that there was still substantial overlap between groups. The isopalisade and (especially) the homogeneous grasses tend to have leaves formed by a larger number of smaller mesophyll cells. The dorsiventral species, and the 12

Table 2. *Average values of leaf attributes measured for 94 species from the Eastern Pamir Mountains*

SLM mg dm^{-2}	A_L dm^2	M_L Mg	L_{thick} μm	D_{mes} cm^{-2}	$V_{mes.cell}$ 10^3 μm^3	V_{chl} μm^3	N_{chl}/M_{cell}	A_{mes}/A_L m^2 m^{-2}
1164	14.87	17317	280	320.49	60.62	29.10	113	16.57
357	3.14	1121	259	25.10	81.50	40.32	63	0.90
550	1.14	627	626	524.29	38.06	75.17	31	13.93
1101	0.24	260	267	1092.92	10.04	33.63	44	24.07
1000	1.00	1000	340	2292.47	8.60	38.47	23	28.77
704	0.69	485	376	482.88	18.49	54.54	48	16.05
880	0.62	546	452	1281.71	14.59	62.86	22	21.12
595	1.38	820	424	156.96	22.99	45.14	66	6.35
690	1.07	740	203	1145.80	5.05	27.25	30	15.24
799	0.55	440	200	1194.55	6.09	39.87	38	25.72
639	49.20	31430	814	256.27	154.64	45.28	56	8.74
1048	1.54	1614	354	622.65	10.71	31.27	56	16.72
753	7.60	5726	438	552.59	31.98	36.11	55	16.20
599	0.78	468	174	2243.93	2.96	32.26	38	41.57
822	7.14	5866	356	271.19	13.77	45.13	32	3.30
601	4.96	2980	378	295.41	32.79	35.15	52	7.91
665	2.18	1449	490	555.19	30.33	64.92	36	15.40
1155	1.82	2102	751	574.87	55.33	47.38	124	45.14
293	0.34	100	345	170.07	17.97	41.30	58	5.72
654	0.14	90	254	265.53	14.57	52.65	59	10.71
409	1.27	519	237	1273.39	3.33	28.14	22	12.48
794	1.05	833	324	1871.81	6.79	42.27	27	29.37
1250	3.41	4262	456	721.03	30.99	38.86	54	21.79
614	19.39	11910	589	639.27	37.22	44.08	39	15.17
779	0.46	360	251	882.17	6.00	23.94	31	10.91
1177	0.19	220	881	414.98	45.86	31.79	69	13.89
579	1.73	90	355	432.15	29.63	66.94	44	15.32
440	0.20	90	338	306.14	21.88	35.43	31	4.95
934	0.93	869	349	706.45	13.97	49.33	51	23.32
663	1.16	770	614	318.39	43.80	54.32	47	10.40
1826	0.21	380	1085	204.74	459.78	33.91	63	6.55
963	0.37	360	1136	96.01	298.19	64.11	185	13.75
1032	0.59	609	1389	47.38	60.18	26.16	214	4.32
440	0.41	180	855	132.91	165.44	31.85	80	5.18
602	7.77	4678	274	3351.98	3.38	26.38	13	19.25
821	3.84	3150	273	5423.59		32.71	28	73.78
521	1.73	901	325	1979.91	1.85	28.91	17	15.09
440	4.90	2156	284	1331.70	3.95	26.73	21	12.19
518	1.54	797	333	2420.26	2.26	29.02	18	19.78
408	5.76	2351	152	1558.01	1.89	21.94	16	9.35
761	0.94	714	1130	389.81	6.83	36.60	39	8.18
415	3.45	1431	243	1033.01	4.31	21.95	34	13.22
492	6.95	3423	238	1189.69	2.92	25.43	35	17.42
364	8.06	2933	298	904.28	8.91	34.55	34	15.69
1017	0.76	772	314	908.20	18.89	50.27	39	23.09
501	4.30	2152	276	1043.45	6.73	43.07	35	21.83
920	9.52	8760	299	750.74	8.79	90.22	40	14.84
424	0.18	76	363	251.66	16.31	65.85	25	4.91
660	0.07	46	455	228.14	31.18	32.01	81	9.03
548	4.09	2241	471	584.43	35.74	30.44	47	13.05
477	7.50	3580	267	724.54	36.19	46.99	32	14.52
685	2.82	1932	365	629.82	13.90	27.98	51	14.44
643	0.29	186	213	1690.55	3.40	33.04	22	18.70
1656	1.38	2280	666	725.36	18.02	65.94	50	28.61
1669	0.02	26	530	1645.41	11.22	39.51	43	39.82
526	3.41	1795	396	364.30	29.96	34.81	35	6.56
762	1.18	899	436	622.45	17.29	40.30	43	15.08
1193	8.11	9671	240	1957.27	3.39	33.70	27	26.67
503	2.23	1121	402	321.19	6.30	25.40	32	8.47
502	5.86	2940	229	221.63		50.24	37	5.33
461	2.04	939	163	65.95	9.28	27.67	31	0.89
959	13.50	12940	420	268.74		30.23	65	8.17
351	1.34	4670	130	728.46	0.50	29.15	12	4.11
445	1.67	744	294	338.34	87.78	56.48	143	34.36
1163	1.27	1477	662	1049.22	13.08	38.18	63	36.38

Table 2 (*cont.*)

SLM mg dm^{-2}	A_L dm^2	M_L Mg	L_{thick} μm	D_{mes} cm^{-2}	$V_{mes.cell}$ 10^3 μm^3	V_{chl} μm^3	N_{chl}/M_{cell}	A_{mes}/A_L m^2 m^{-2}
813	2.75	2240	443	611.59	11.43	39.59	31	10.59
1164	70.89	82538	687	682.55	19.79	64.82	42	22.14
391	0.20	78	377	172.16	39.78	49.37	45	5.06
763	0.16	120	499	180.22	32.46	72.65	95	14.48
721	1.93	1390	326	332.36	13.06	48.63	66	14.19
1135	0.59	670	631	551.83	19.84	37.03	60	17.65
1062	13.38	14202	387	382.10	21.74	39.89	69	14.89
395	2.73	1078	377	319.08	44.85	44.36	54	10.35
822	5.18	4258	345	406.97	17.99	37.40	108	23.71
587	0.09	50	584	121.51	14.77	37.84	67	4.44
685	2.32	1589	325	229.81	29.83	30.80	97	10.55
461	1.36	626	379	352.29	39.29	39.21	81	15.87
616	1.82	1122	407	620.06	33.41	43.13	45	16.70
547	1.68	919	413	309.42	36.18	40.21	92	16.24
349	1.02	356	231	480.98	3.07	26.77	35	7.25
880	0.64	563	135	4132.94	1.30	33.86	11	22.22
781	4.95	3867	261	1858.73	6.16	31.68	24	21.89
812	1.97	1600	163	1338.91	3.07	30.08	19	11.61
1274	1.60	2039	237	1482.79	2.96	24.40	49	29.51
1017	4.09	4159	282	1345.08	9.62	33.22	41	27.41
1288	0.98	1267	212	4063.42	1.89	29.34	22	41.14
890	0.16	140	177	3024.16	2.89	39.74	23	38.39
862	0.45	390	443	184.58	58.81	43.56	84	9.31
701	2.42	1700	304	356.11	52.19	34.80	73	13.38
738	3.58	2640	466	913.89	35.49	41.66	49	25.82
1100	0.18	198	291	503.65	21.55	36.87	54	14.68
514	0.43	222	411	179.98	81.20	58.00	50	6.48
625	0.70	437	332	767.91	11.18	35.50	40	16.01
1608	0.66	1061	803	831.60	72.91	52.47	109	61.45

Variables: specific leaf mass (SLM); leaf surface area (A_L), mass (M_L) and thickness (L_{thick}); mesophyll density (D_{mes}), volume of average mesophyll cell ($V_{mes.cell}$) and average chloroplast (V_{chl}); number of chloroplasts per mesophyll cell (N_{chl}/M_{cell}); mesophyll area per leaf area (A_{mes}/A_L).

species having other types of mesophyll arrangement, tended to have fewer, larger mesophyll cells, thicker leaves and more, larger chloroplasts per mesophyll cell.

DISCUSSION

Size versus number

In this study we found that mesophyll cell size and number per cm^2 were strongly but negatively correlated (Fig. 1). A possible biological explanation for this size compared with number trade-off comes from the observation that leaf thickness was positively correlated with mesophyll cell volume, but negatively correlated with mesophyll cell density. Since lamina thickness will be determined largely by the average size of the mesophyll cells and the number of layers of such cells within the leaf, this negative correlation probably arises from the functional requirement for leaf thickness to be constrained by environmental conditions, especially by irradiance level. The thickness of the leaf lamina will determine the efficiency with which photons can be trapped and used by the leaf. Leaves with larger mesophyll cells, and therefore more and larger chloroplasts per cell, will tend to absorb more incoming photons, leaving less residual energy available to penetrate into other mesophyll cells deeper in the leaf lamina. This restricts the number of layers of mesophyll cells that can maintain a positive C gain. If lamina thickness is constrained within set limits, then the leaf must trade off mesophyll size and density. The effect of increasing the number (and therefore decreasing the size) of the mesophyll cells would be to increase the total surface area of the mesophyll. This would affect photosynthetic rate, as the ratio of the surface area of the mesophyll cells to the surface area of the leaf (A_{mes} : A) is correlated with the rate of CO_2 assimilation (Nobel *et al.*, 1975; Longstreth & Nobel, 1979; Nobel & Walker, 1985; Patton & Jones, 1989).

Such a trade-off with respect to the size and number of chloroplasts was not observed within mesophyll cells; larger mesophyll cells had both more and larger chloroplasts. No explanation suggests itself for this, but the total volume of chloroplasts within a mesophyll cell is a very small proportion of the total mesophyll cell volume. The question of the relative advantages and disadvantages

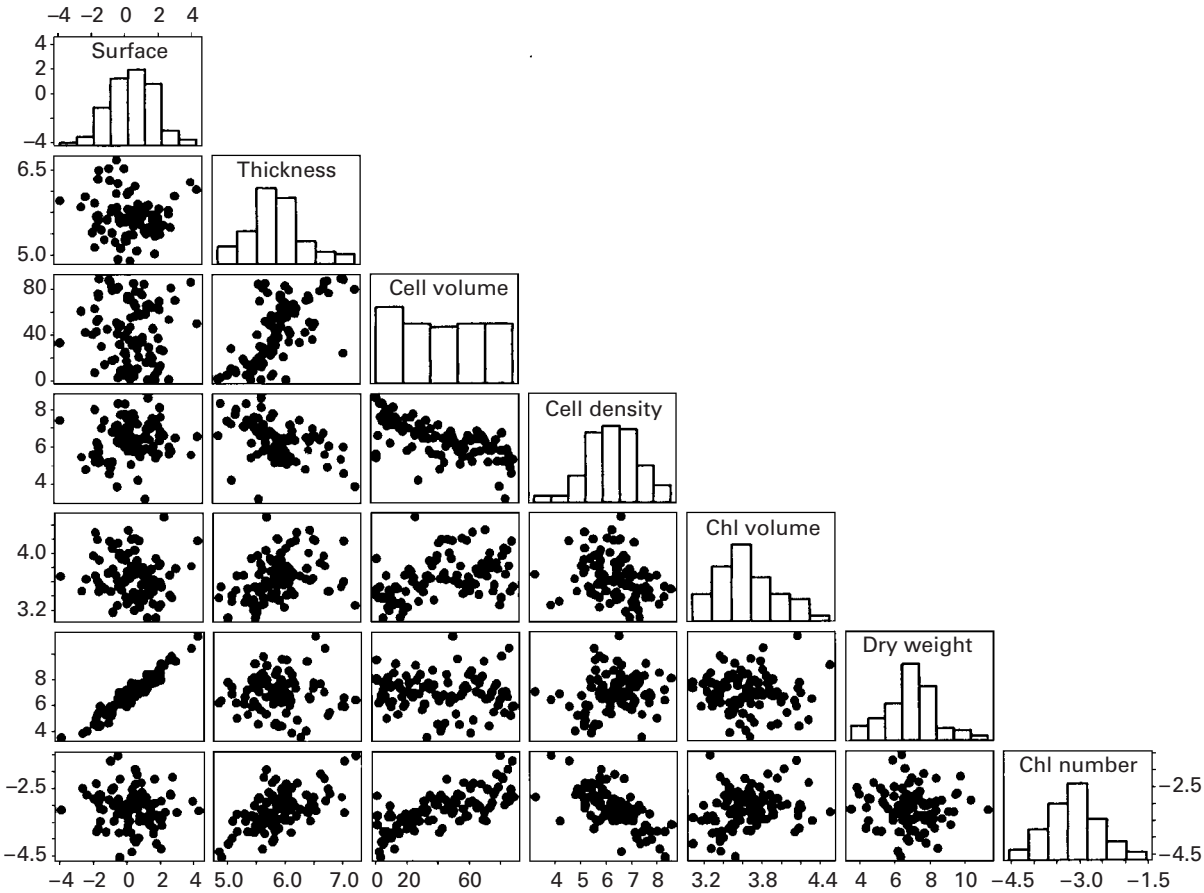

Fig. 1. Scatterplot matrix of seven leaf attributes ($\log_e$ transformed) based on means of 94 species of field-collected plants. Diagonal panels show the distribution of each variable; off-diagonal panels show scatterplots: *x* axis, column variable; *y* axis, row variable.

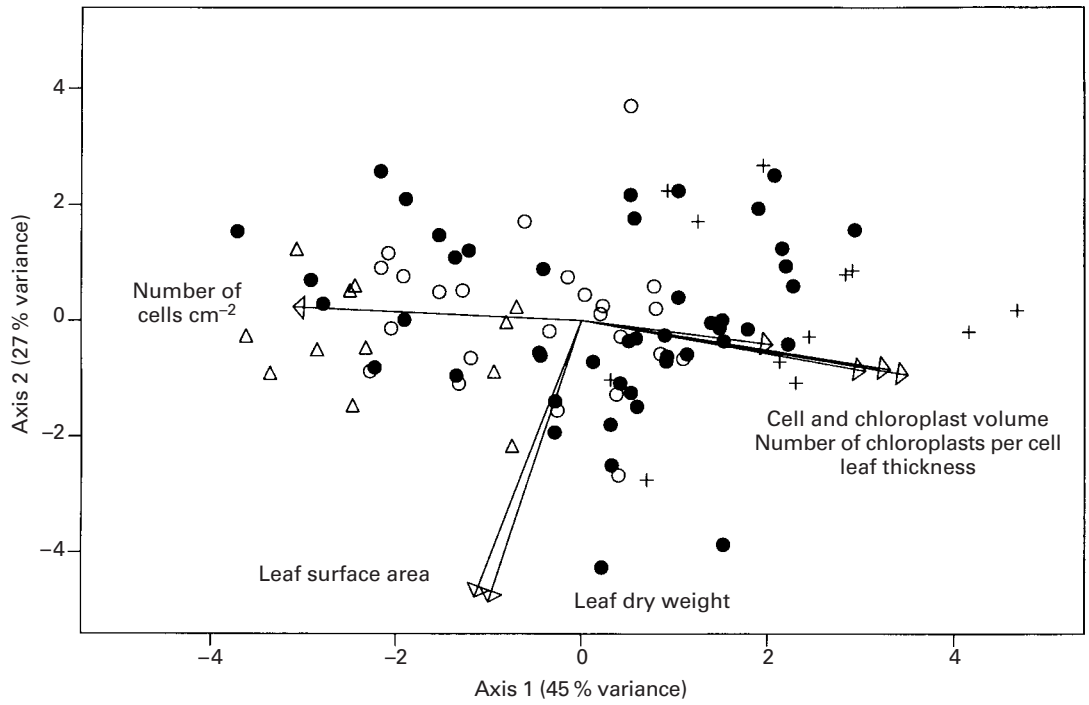

Fig. 2. Biplot showing the species scores (symbols) of 94 species of field-collected plants on the first two axes of a principal components analysis of seven leaf attributes ($\log_e$ transformed). Loadings of the leaf attributes (multiplied by 7 for visual clarity) on the first two axes are shown by arrows. The three different mesophyll types are: dv, dorsiventral (closed circles); ip, isopalisade (open circles); hg, homogeneous grasses (open triangles); other types (crosses).

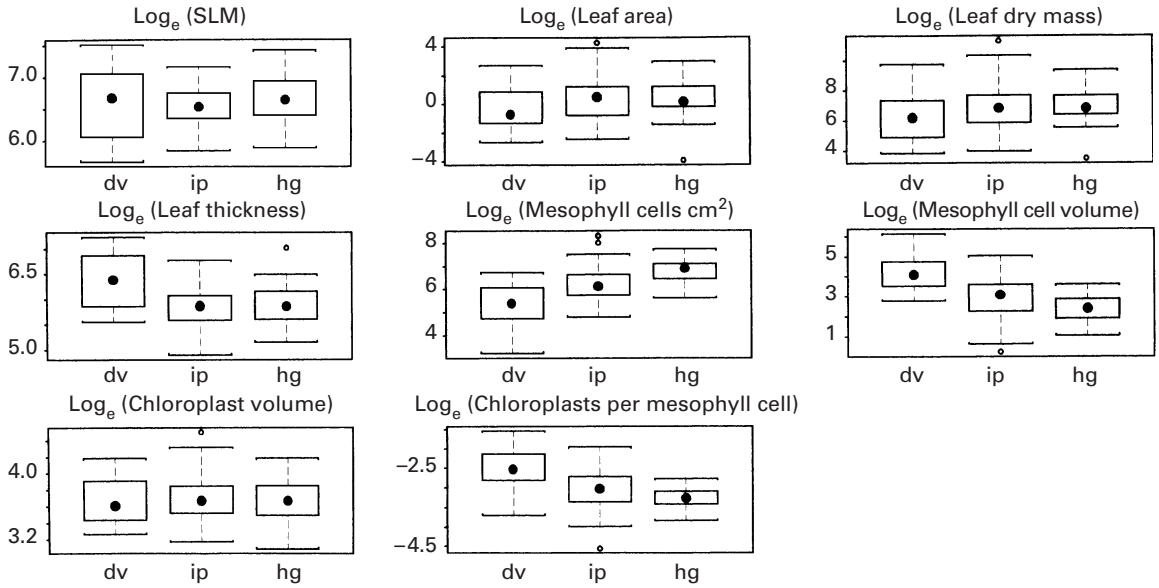

Fig. 3. Box plots of seven leaf attributes ($\log_e$ transformed) grouped according to mesophyll structure (dv, dorsiventral; ip, isopalisade; hg, homogeneous grasses). Solid dots show the mean, the box gives ± 1 quantile, bars show 95% quantiles, and outliers are shown by open circles. Significant differences in the means exist between groups for every variable.

of different combinations of mesophyll cell and chloroplast size versus number in different environmental contexts is important, but relatively unstudied. For example, given the necessary scaling of surface area to volume of such chloroplasts, larger chloroplast size would probably affect the diffusion of CO_2 into chloroplasts.

Size versus number in relation to specific leaf mass

The SLM is the product of leaf thickness and leaf tissue density. Leaf tissue density (leaf dry mass per volume of leaf tissue, not including intercellular spaces) is often measured as the proportional water content of the leaf; that is, the proportion of leaf fresh weight that is due to leaf water (Garnier & Laurent, 1994; Shipley, 1995). This is because most of the volume of most leaf cells (except for xylem or those forming structural tissues) is occupied by cytoplasm. We can therefore expect that leaf tissue density will be strongly affected by the size and number of mesophyll cells. Most of the mass of a mesophyll cell is concentrated in its cell wall, and is therefore proportional to its surface area and cell wall thickness. Most of the water in the cell is in the cytoplasm, and the mass of water is proportional to the volume of the mesophyll cell. It follows that larger mesophyll cells would generally have a larger proportion of water (cytoplasm) to dry mass (cell wall). This means that larger mesophyll cells would contribute to a larger proportional water content and a lower leaf tissue density. Increasing the number of such cells would not change this proportionality.

Although conceptually different, leaf thickness and leaf tissue density are not biologically independent. For instance Meziane & Shipley (1999) grew 22 herbaceous species typical of open, sunny, lowland habitats, under controlled conditions in hydroponic sand culture in four different environments: high (1100) and low (200) irradiance (μmol m^{-2} s^{-1} PAR) crossed with a high (1:1) and low (1:6 dilution of the hydroponic solution) nutrient concentration. They found that mesophyll and lamina thickness increased with increasing irradiance and with increasing nutrient supply. Conversely, leaf water content decreased with increasing irradiance, but increased with increasing nutrient supply. Lamina thickness and leaf water content were negatively correlated across species at low irradiance but the two were largely uncorrelated at high irradiance. This is consistent with the explanation given above. At the low photosynthetic photon fluence density (PPFD) (200 μmol m^{-2} s^{-1} PAR) self-shading within the leaf would have constrained cell layering, generating the negative correlation; presumably the high PPFD used in that experiment largely overcame self-shading within the leaves.

Modelling specific leaf mass

The path model of SLA proposed by Shipley (1995) essentially expressed SLA as leaf thickness and leaf tissue density, measured by proportional water content. The model is shown in Fig. 4a in qualitative form (excluding the thickness of the protruding midvein for which we have no measures in this paper); note that the original model dealt with specific leaf area, not mass, so the signs of the path coefficients are reversed in Fig. 4a. This model was shown to provide a good fit to data obtained from field-collected herbaceous plants typical of open sunny habitats in south-eastern Canada. As previously

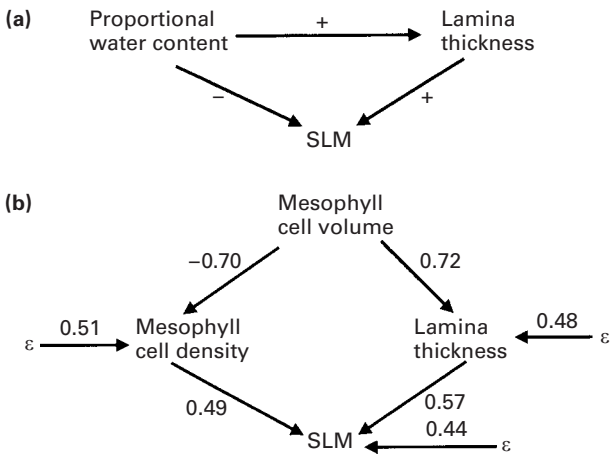

Fig. 4. (a) Path model proposed by Shipley (1995). (b) First path model proposed in this study. Model parameters are based on standardized variables. Error variables (ε) have a variance of 1. This model produced a good fit to the empirical data.

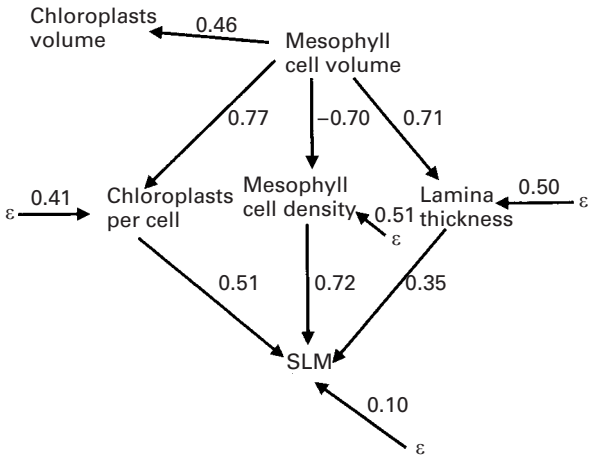

Fig. 5. Second path model proposed in this study. Model parameters are based on standardized variables. Error variables (ε) have a variance of 1. This model produced a good fit to the empirical data.

argued, leaf proportional water content will be partly determined by the average volume of the mesophyll cells. An effect from mesophyll volume to mesophyll density was included because there is a trade-off between average mesophyll cell volume and the number of these cells per cm^2 cross-section. Therefore the path model shown in Fig. 4b was proposed.

This model produced a good fit to the data ($\chi^2 = 3.50$, 2 df, $P = 0.17$) and, using the Satorra–Bentler chi-square statistic (S–$B\chi^2$) which corrects for non-normality (Bentler, 1995), indicates an even better fit (S–$B\chi^2 = 2.02$, 2 df, $P = 0.36$). This model points to the central role of mesophyll cell volume in relating the measured variables to SLM. Increasing mesophyll cell volume increases leaf thickness but decreases the number of mesophyll cells in cross-section. This trade-off between size and number results in leaf thickness being negatively correlated with mesophyll cell density ($r = -0.44$). The SLM increases both with increasing leaf thickness and with increasing mesophyll cell density. This means that increasing mesophyll cell volume increases SLM by increasing leaf thickness, but decreases SLM by decreasing mesophyll cell density. The result is that the overall correlation between mesophyll cell volume and SLM is almost zero ($r = 0.07$).

Although cell dry mass would be mostly determined by cell wall mass, the mass of cellular organelles such as chloroplasts would also contribute. From the empirical data, it is known that both chloroplast number and size are positively correlated with mesophyll cell volume. Fig. 5 therefore presents an enlarged version of the path model in Fig 4b. This model also gives a good fit to the data (S–$B\chi^2 = 9.98$, 8 df, $P = 0.27$) and indicates that an increasing number of chloroplasts per mesophyll cell also increases SLM. Again, average mesophyll cell volume is a key variable in the model. Although one might think that chloroplast volume and the number of chloroplasts per mesophyll cell should affect SLM, no empirical evidence was detected for this. Including a path from chloroplast volume to SLM in the model shown in Fig. 5 does not affect the overall fit of the model, but this path is not significantly different from zero ($t = 0.16$, $P = 0.87$). All of these path models should be interpreted simply as hypotheses that are consistent with the available data. Independent data from species occurring in different habitats are required to provide strong tests of the models.

The ecological relevance of specific leaf mass and its components

Shipley's (1995) field data, obtained from herbaceous species from open sunny habitats typical of south-eastern Canada, had an average SLM of 362 mg dm^{-2}, with most being between 252 and 645 mg dm^{-2}. Typical values of herbaceous species from the forest understorey in south-eastern Canada (unpublished) range from 169 to 305 mg dm^{-2} with an average of 225 mg dm^{-2}, and for 160 understorey dicot species from the Middle Urals (boreal forest) the modal class for this parameter was 200–350 mg dm^{-2} (Barinov, 1988; Pyankov *et al.*, 1998; V. I. Pyankov *et al.*, unpublished). Körner & Diemer (1994) showed the average SLM in their mountain plants increased from 436 to 606 mg dm^{-2} as altitude increased from 600 to 2600 m in the Austrian Alps. The modal class of SLM for arctic plants (*c.* 100 species) was 400–600 mg dm^{-2} (Barinov, 1988). By contrast, the SLM values recorded for the species in this paper, growing in extremely cold and dry habitats in the Eastern Pamir Mountains, average 706 with the first and third quartiles being 518 and 952 mg dm^{-2}. The fact that no significant differences in SLM or other leaf attributes were detected in

these Eastern Pamir species when combined into ecological groups (mesophytes, xerophytes or intermediates) or degree of rarity, is probably partly because all of the species are adapted to the extreme conditions of this area. It may also be due to the fact that there was substantial heterogeneity among species within these groups, because every ecological or rarity group included plants belonging to different life forms, altitudinal ranges and taxonomic affiliations (Pyankov & Kondratchuk, 1998). The one classification that did produce significant differences was that based on the organization of the mesophyll. Species with dorsiventral palisade had larger average mesophyll cells. Consistent with the path diagrams, this translated into thicker leaves with fewer mesophyll cells per square centimetre and more chloroplasts per mesophyll cell, but only small differences in SLM. Because of the scaling of surface area to volume and the partial trade-off between mesophyll cell size and number, dorsiventral species tend to have a lower $A_{mes} : A$ ratio. This would increase CO_2 mesophyll resistance, resulting in poorer drought tolerance. What, then, might be the adaptive advantage of the dorsiventral organization with its large mesophyll cells? This cannot be answered, but increased irradiance increases the development of the palisade and can even change leaves from dorsiventral into isopalisade, as demonstrated on leaves in different parts of the crown (Tselniker, 1978) or plants developing in spring or summer as irradiance increases (Goryshyna, 1989). Perhaps these species are trading off drought tolerance and the ability to photosynthesize efficiently at high PPFD. If this is the case then the different paths from mesophyll cell volume to SLM may represent these different selective pressures. Further research is needed to test these hypotheses.

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